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This study utilized the developed in vitro models and the identified biochemical and cellular markers to examine the induction of human mammary carcinogenesis by the environmental carcinogen Benzo(α)pyrene (BP), and its modulation by selected naturally-occurring tumor inhibitors. In the human mammary explant culture system BP treatment increased Ras p21-GTP binding, and replicative DNA synthesis, and altered the metabolism of 17ß-estradiol (E_2). Treatment of BP-initiated explant cultures with n-3 polyunsaturated fatty acid eicosapentaenoic acid or indole-3-carbinol inhibited replicative DNA synthesis and increased C2/C16α-hydroxylation of E_2, thereby down-regulating BP-induced biomarker expression. In the human mammary epithelial cell culture system treatment with BP resulted in increased purine nucleotide adduct formation, arrest of the cell cycle in G2 + M phase and abrogation of cellular apoptosis. Treatment of BP-initiated cells with the soy isoflavone genistein inhibited the S phase and induced apoptosis. These results demonstrate that noninvolved human mammary tissue responds to the carcinogenic insult by perturbed expression of biochemical and cellular markers prior to tumorigenic transformation, and biomarker expression is down-regulated by the naturally-occurring tumor inhibitors. Thus, the biochemical and cellular markers represent quantitative endpoints for chemoprevention of human mammary carcinogenesis.

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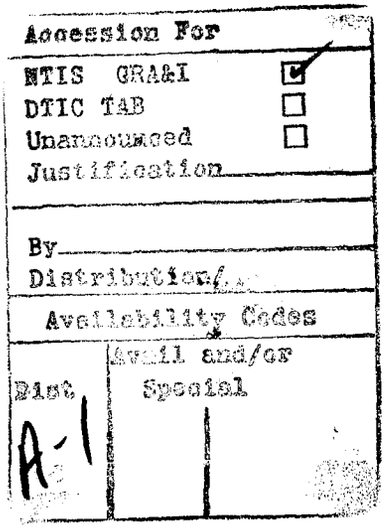

Mitin T. Telang 07/30/95

PI - Signature Date

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I. INTRODUCTION

This report summarizes the proposed experiments conducted during the funding of USAMRDC IDEA award # DAMD 17-94-J-4208, for the first year. The experiments have utilized the explant culture and cell culture systems established from non-involved human mammary tissue to examine i) the effects of prototypic chemical carcinogens and oncogenes that are associated with preneoplastic transformation, and ii) whether selected naturally-occurring tumor inhibitors modulate the effects of chemical carcinogens and oncogenes.

1. Nature of the problem: In the multistage development of mammary cancer, early-occurring events of initiation and promotion leading to preneoplastic transformation provide useful molecular, biochemical and cellular markers to identify relative risk for developing the disease (1,2). The American Cancer Society has estimated about 189,000 new breast cancer cases and about 47,800 cancer-related deaths in 1995 (3). Identification and validation of specific and sensitive biomarkers for risk and efficacious prevention therefore remains a high priority area of research.

The in vitro models for rodent mammary carcinogenesis have displayed the induction of preneoplastic transformation in response to such prototypic initiators as chemical carcinogens (4,5), oncogenes (6,7) and transforming retrovirus (8,9). The extent of induction of preneoplastic transformation is quantified at molecular, biochemical and cellular levels

using specific biomarkers as endpoints (10,11). A comparable model for human mammary carcinogenesis and its prevention, however, remains to be developed.

2. **Background of previous work:** Our previous studies on the explant cultures of human mammary terminal duct lobular units (TDLU) have shown that i) the epithelial component exhibits persistent cell turnover, i.e., detectable replicative DNA synthesis in a chemically defined, serum-free medium ii) the explanted tissue metabolizes 17ß estradiol (E_2) via the major metabolic pathways operative in vivo, and iii) prototypic rodent mammary carcinogens induce up-regulation of ras-mediated signal transduction (12-14). The data generated from these studies provide evidence for the validity of replicative DNA synthesis, cellular metabolism of E_2 and Ras p21-GTP binding as endpoint biomarkers for human mammary carcinogenesis.

3. **Purpose of the present work:** The ongoing and planned experiments are designed to obtain evidence for the clinical relevance of the developed biomarkers for effective chemoprevention of human mammary carcinogenesis. The ongoing studies have utilized the explant culture system from human mammary TDLU and the cell culture system from reduction mammoplasty-derived 184-B5 cells. This comparative approach is expected to elucidate i) the role of stromal component on induction and modulation of mammary carcinogenesis, and ii) the extent of direct participation of the target mammary epithelial cells in the process of carcinogenesis and chemoprevention.

4. Methods of approach: The techniques used for maintenance of TDLU explant cultures and for cell cultures of 184-B5 and 184-B5/HER cell lines are essentially similar to those reported in the previous studies (12-14,16). The basal medium consists of DME/F12 for explant culture and MEM/KBM for cell culture. The media are supplemented with antibiotics, L-glutamine, hormones and growth factors. Thus, growth in a chemically defined serum-free milieu provides a means of analyzing the direct effects of initiators and/or modulators for mammary carcinogenesis under stringently controlled culture conditions.

The quantitative parameters utilized to examine the effects of initiators and modulators of carcinogenesis include replicative DNA synthesis (RDS) assay, Ras-p21-GTP binding assay, radiometric assay for E_2 C16α-hydroxylation and C2-hydroxylation, anchorage-dependent growth (ADG) assay and flow cytometric assay for cellular apoptosis. The methodology for all these assays is published (5,9-11).

Initial dose response experiments identified the maximally effective, highest non-toxic doses of chemical carcinogens and naturally-occurring chemopreventive agents. Optimum concentrations and durations of exposure of the individual agents have been used in the completed experiments. The relative extent of perturbation of biochemical or cellular endpoint biomarker by chemical carcinogen or oncogene provides a measure of induction of preneoplastic transformation. The extent of inhibition of the perturbed biomarkers provides the evidence for effective primary prevention (see AT-1 for the developed biomarker assays).

II. PROGRESS REPORT (07/01/94 - 06/30/95):

The experiments completed during the first year of funding utilized the TDLU explant culture system and the 184-B5 cell culture system to test the following hypothesis: **Acute, direct effects of the environmental carcinogen benzo(α)pyrene (BP) are down-regulated by selected naturally-occurring dietary compounds, as evidenced by inhibition of clinically relevant biochemical and cellular endpoint biomarkers.**

The endpoints selected as quantitative parameters are surrogate markers for initiational and promotional events associated with preneoplastic transformation. The present experimental approach therefore provides clinically relevant leads for effective primary prevention of human mammary carcinogenesis via inhibition of preneoplastic transformation.

1. Experiments on TDLU explant cultures: The experiments utilizing the TDLU explant culture model were designed to examine i) responsiveness of the non-involved target tissue to an environmental carcinogen benzo(α)pyrene (BP), and ii) modulatory influence of selected naturally-occurring agents on the acute effects of BP.

i) Effect of BP on explant cultures of human mammary TDLU: For the experiments to examine acute direct effect of the environmental carcinogen BP, explant cultures were prepared and maintained in a chemically defined, serum-free medium following the previously published methods (12-14). The cultures were treated with the previously identified nontoxic dose of 10 μg/ml BP for 24 hr. The effect of BP was evaluated by

determining the relative extent of Ras p21-GTP binding and 16α-hydroxyestrone (16α-OHE_1) formation by metabolism of 17ß-estradiol (E_2) via the C16α-hydroxylation pathway (10,11,13,14). The experiment presented in AF-1 demonstrated that exposure to BP results in a 4-fold increase ($p = 0.001$) in binding of Ras p21 to GTP, and a 6-fold increase ($p = 0.001$) in 16α-OHE_1 formation, relative to that observed in solvent-treated control cultures. The two quantitative parameters represent markers for Ras-mediated signal transduction and cellular metabolism of E_2. Experiments on the mouse mammary explant and cell culture systems have shown that exposure to chemical carcinogen or transfection with Ras oncogene results in up-regulated Ras p 21-GTP binding activity and E_2 metabolism via the C16α-hydroxylation pathway (10,11). Taken together, these results suggest that the two biochemical endpoints represent useful markers for target tissue response to initiators of mammary carcinogenesis.

ii) **Effect of naturally-occurring dietary agents on BP-induced carcinogenesis:** The modulatory effect of naturally-occurring n-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA) and indole-3-carbinol (I3C) was examined by determining the alteration in biochemical markers that are up-regulated in response to carcinogen treatment. EPA and I3C were selected as the test compounds because of their documented tumor inhibitory effects (17,18), and their ability to down-regulate rodent mammary carcinogenesis <u>in vitro</u> (1,2,9,11). For these experiments the TDLU explant cultures were treated with 10 μg/ml BP for 24 hr. The BP-initiated cultures were then maintained in the presence of 5 μg/ml EPA or I3C for subsequent 14 days. The cultures were pulse labeled with 5 μci/ml ^{3}H-

thymidine for the last 24 hr. prior to harvest, and the extent of ^{3}H-thymidine uptake was determined by trichloroacetic acid (TCA) precipitable ^{3}H radioactivity in the tissue homogenates (5,9,12). ^{3}H-thymidine uptake represented a measure of replicative DNA synthesis that was indicative of the proliferative status of TDLU explants. The data presented in AF-2 demonstrate that treatment of TDLU explants with BP resulted in a 104% increase ($p = 0.001$) in ^{3}H-thymidine uptake, relative to that observed in the DMSO-treated solvent control. This increase is indicative of hyperproliferation. Treatment of BP initiated cultures with EPA and I3C resulted in a 40% and a 47% inhibition ($p = 0.001$) in ^{3}H-thymidine uptake, relative to that observed in cultures treated with BP alone. These data suggest that EPA and I3C effectively down-regulate BP-induced hyperproliferation.

Experiments on the mouse mammary epithelial cells have shown that exposure to chemical carcinogen or transfection with oncogene results in an increase in E_2 16 α-hydroxylation with a concomitant decrease in E_2 C2-hydroxylation (1,10,14). This alteration in cellular metabolism of E_2 leads to a decrease in the C2/C16α hydroxylation ratio. To examine whether treatment of TDLU with BP influences E_2 metabolism, and whether this metabolic alteration is modulated by EPA and I3C, the 12 day old TDLU explants treated with DMSO, BP, BP + EPA and BP + I3C, were incubated with [^{3}H-C2]E_2 and [^{3}H-C16α]E_2 for the last 48 hr. The medium was processed for determining E_2 metabolism via C2- and C16α-hydroxylation pathways, using the radiometric assay (10,11,13,14). The extent of E_2 metabolism was expressed as C2/C16α hydroxylation ratio. The data presented in AF-3 demonstrate that exposure to BP results in about 80% decrease in C2/C16α-hydroxylation

ratio ($p < 0.0001$) relative to that observed in DMSO-treated control cultures. Furthermore, subsequent treatment of BP-initiated cultures with EPA and I3C resulted in almost complete abrogation of the decrease induced by BP.

In conclusion, our recently completed experiments on the human mammary explant culture model have demonstrated that i) acute direct exposure of TDLU explants to the environmental carcinogen BP induces up-regulation of ras-mediated signal transduction and of 16α-OHE_1 formation; and enhancement of cellular proliferation, ii) the perturbed biochemical and cellular markers in the BP-initiated tissue are inhibited by selected naturally-occurring tumor inhibitors. It is, however, noteworthy that cellular heterogeneity intrinsic to the explant culture model (presence of non-epithelial cell types) precludes experiments designed to elucidate a direct effect of initiator or modulators on the transformation-sensitive target epithelial cell.

2. **Experiments on human mammary epithelial cell cultures:** In an effort to overcome the technical limitations of cellular heterogeneity present in the mammary explant culture model, experiments were performed on reduction mammoplasty-derived 184-B5 cells. The completed experiments were designed to i) compare the growth kinetics of parental 184-B5 and HER-2/neu oncogene-initiated 184-B5/HER cells, ii) examine the acute, direct effects of the environmental carcinogen BP on 184-B5 cells, and iii) determine dose-response of selected naturally-occurring agents (-)-epigallocatechin gallate (EGCG), indole-3-carbinol (I3C) and genistein (GEN) for growth inhibition of 184-B5 and 184-B5/HER cells.

i) **Anchorage-dependent growth of 184-B5 and 184-B5/HER cells:** The parental as well as oncogene-initiated human mammary epithelial cells exhibited progressive time-dependent increase in growth. The 184-B5 cells exhibited a population doubling time (PDT) of 32.8 ± 1.6 hr and about a 24-fold increase at day 9 post seeding. In contrast, 184-B5/HER cells showed a 34% decrease in PDT relative to that in 184-B5 cells, and a 38-fold increase in cell number 9 days post seeding (AT-2). These results suggest that over-expression of HER-2/neu oncogene induces aberrant hyperproliferation in non-tumorigenic human mammary epithelial cells.

ii) **Effect of BP on 184-B5 cells:** Acute, direct effects of BP were evaluated by determining the extent of growth inhibition and of carcinogen-DNA adduct formation. Initial dose-response studies revealed a clear concentration-dependent inhibition of growth. Thus, a single 24 hr treatment with 1.0 and 10 μg/ml resulted in persistent 79.7% and 97.1% inhibition in the number of surviving cell population 7 days post treatment. These data are indicative of an acute antiproliferative effect of BP on 184-B5 cells.

The genotoxic response of 184-B5 cells to BP was examined using the [^{33}P] post labeling assay specific for carcinogen-DNA adduct formation (5,15). A 24 hr. treatment of 184-B5 cells to 10 μg/ml BP revealed the presence of BP diolepoxide: deoxyguanosine (BPDE:dGuo) and BP diolepoxide:deoxyadenosine (BPDE:dAdo) adducts that were undetectable in parallel cultures treated with DMSO, the solvent for BP (AT-3).

BP is a metabolism-dependent procarcinogen requiring P450-dependent oxidative reactions for generation of genotoxic and mutagenic derivatives. Our ability to detect BP-induced negative growth regulation and purine nucleotide adduct formation in 184-B5 cells suggests that the non-tumorigenic mammary epithelial cells may have the intrinsic metabolic competence to generate DNA damaging metabolites from BP.

Apoptosis plays an important complementary role with proliferation to maintain cellular homeostasis (19). The positive growth regulation by initiators of carcinogenesis may also impact upon regulated apoptosis. This aspect was examined by determining whether treatment of 184-B5 cells with BP alters cellular apoptosis. Cell cultures at ≈ 80% confluence were treated with 10 μg/ml BP. Parallel cultures treated with 0.1% DMSO (solvent) represented the controls. Cell suspensions form the two treatment groups were stained with propidium iodide and processed for cell cycle analysis by flow cytometry using fluorescence-assisted cell sorter (FACS). The data presented in AT-4 and AF-4 show that in confluent cultures of 184-B5, treatment with BP results in arrest of the cycling cells in the G2 + M phase of the cell cycle, and in abrogation of sub GO apoptotic peak. Furthermore, simultaneous treatment of 184-B5 cells with BP + GEN enhances the G2 + M arrest and induces sub GO apoptotic peak. The possible mechanisms of BP-induced and GEN-induced alterations in cell cycle are being investigated in the ongoing studies. In addition, effects of I3C and EGCG are being examined.

iii) Negative growth regulation in human mammary 184-B5 and 184-B5/HER cells by naturally-occurring phytochemicals: Since the experiments described in Section 2 (ii) have demonstrated specific proliferative differences in 184-B5 and 184-B5/HER cells, it was of interest to examine whether the parental and the oncogene-initiated cells differ in their responsiveness to the selected naturally-occurring phytochemicals that inhibit rodent mammary tumorigenesis. The maximally nontoxic doses of EGCG, I3C and GEN identified by initial dose-response experiments, were used. The data presented in AT-5 demonstrate that Ic_{50} values of the three agents were at least 3-10-fold higher for growth arrest of 184-B5/HER cells relative to that of 184-B5 cells. The possible mechanisms for the growth arrest by EGCG, I3C and GEN are being identified at present.

In conclusion, the experiments conducted using the human mammary epithelial cell culture model have demonstrated that early-occurring initiational or post-initiational events in mammary carcinogenesis are quantified using the specific and sensitive biomarkers, and that down-regulation of the perturbed biomarkers represents useful endpoints for efficacy of naturally-occurring tumor inhibitors.

III. GENERAL CONCLUSIONS:

The recently completed experiments, together with the ongoing projects, provide evidence for the applicability of the in vitro human mammary carcinogenesis models as assay systems to identify potential carcinogens, and evaluate the chemopreventive efficacy of

naturally-occurring phytochemicals. These experiments, together with our observations on the rodent in vitro model (1,5,10,11,20,21) demonstrate that the biomarker endpoints are broadly applicable for experimental and human mammary carcinogenesis. Thus, the current research approach should validate a human tissue-derived assay for screening potentially efficacious compounds for prevention of human mammary carcinogenesis. Promising agents could then be tested in conventional clinical trials.

An important aspect of the future studies is to elucidate possible mechanisms of action of the phytochemicals. The molecular, biochemical and cellular biomarker assays represent important means for identifying cellular targets for preventive efficacy,

It is conceivable that phytochemical-induced growth arrest of initiated (preneoplastic) phenotype may be the end result of multiple intermediate events. A systematic analysis of inhibition of human mammary carcinogenesis, therefore, should provide important leads for clinically relevant preventive interventions.

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Appendix Material

AT-1: Developed biomarker assays for primary and secondary prevention

Human mammary explant culture system

AF-1: Effect of BP on Ras p21-GTP binding and 16α-OHE_1 formation.
AF-2: Effect of EPA and I3C on replicative DNA synthesis.
AF-3: Effect of EPA and I3C on C2/C16α-hydroxylation ratio of E_2.

Human mammary cell culture system

AT-2: Anchorage-dependent growth in 184-B5 and 184-B5/HER cells.
AT-3: DNA adduct formation in 184-B5 cells.
AT-4: Cell cycle analysis of 184-B5 cells.
AF-4: Modulation of apoptosis in 184-B5 cells.
AT:5: Regulation of anchorage-dependent growth in 184-B5 and 184-B5/HER cells.

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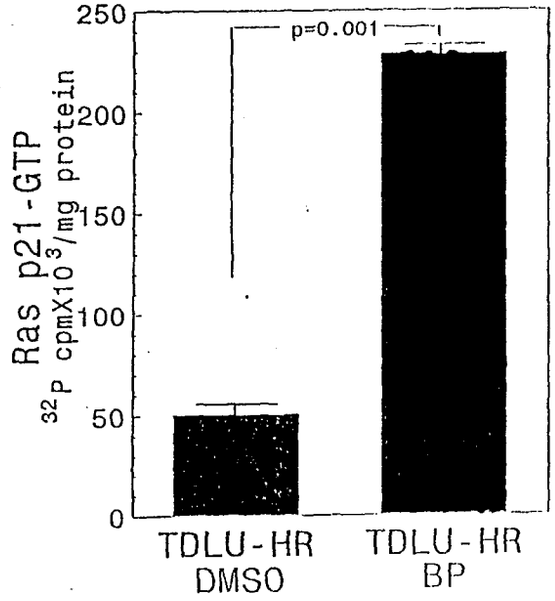
INTERMEDIATE BIOMARKER ASSAYS FOR PRIMARY AND SECONDARY PREVENTION

Biomarker	Assay	Endpoint
molecular	oncogene expression	Southern, Northern and Western blot analysis for DNA, RNA and protein. Ras p21-GTP, myc p60-TTP assay
biochemical	E_2 metabolism	E_1, 16α-OHE_1, 2-OHE_1 formation
	unscheduled DNA synthesis	HU-insensitive thymidine uptake
	HGPRT mutagenesis	TG-insensitive thymidine uptake. HX uptake
cellular	colony forming efficiency anchorage-dependent growth	TG^r colony formation anchorage-dependent colony formation
	anchorage-independent growth	anchorage-independent colony formation
	tumorigenicity	incidence and latency of palpable tumors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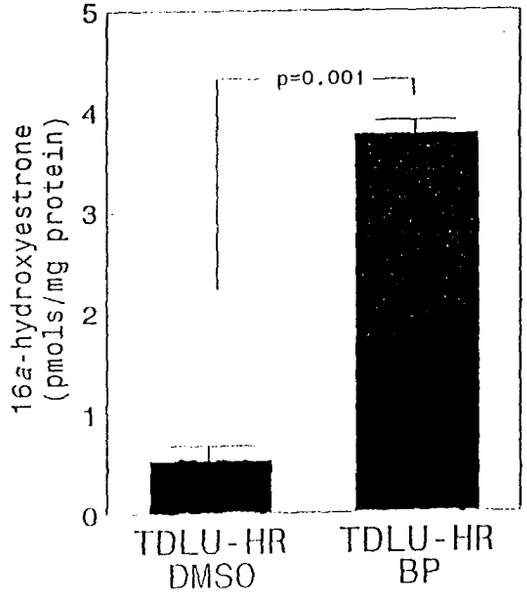
^{32}P cpmX10^{3}/mg protein
250
200
150
100
50
0
p=0.001
TDLU-HR
DMSO
TDLU-HR
BP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(pmols/mg protein)
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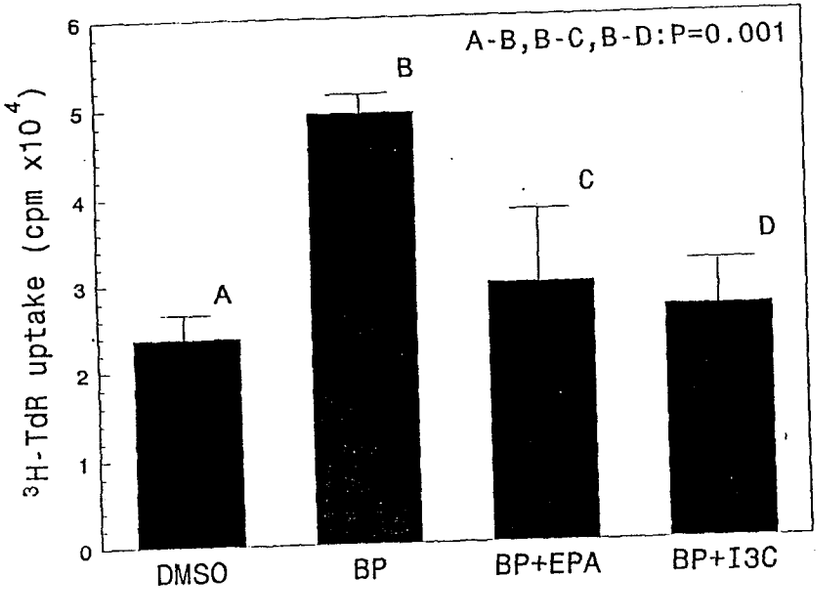
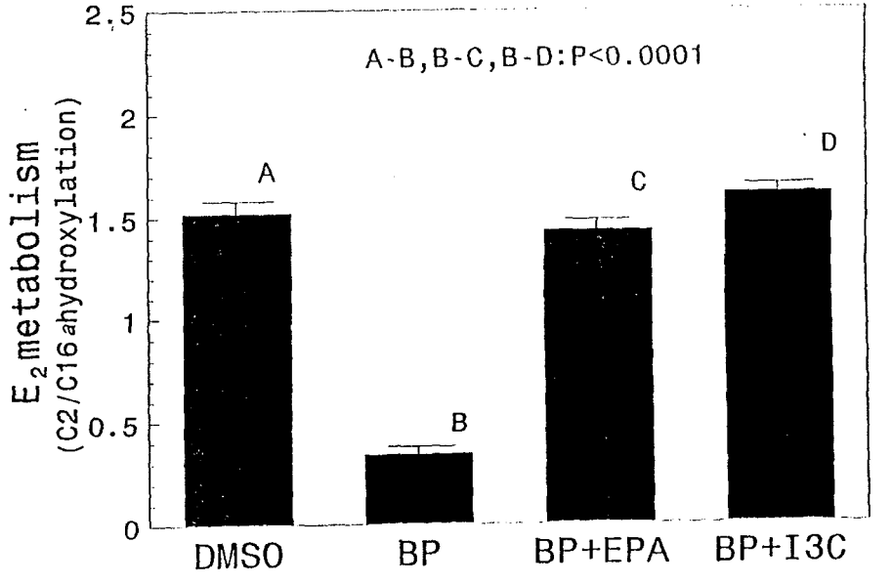
BP


^{3}H-TdR uptake (cpm x10^{4})
6
5
4
3
2
1
0
A-B,B-C,B-D:P=0.001
A
B
C
D
DMSO
BP
BP+EPA
BP+I3C


E$_2$metabolism
(C2/C16ahydroxylation)
2.5
2
1.5
1
0.5
0
A-B,B-C,B-D:P<0.0001
A
B
C
D
DMSO
BP
BP+EPA
BP+I3C

AT-2

GROWTH CHARACTERISTICS OF HUMAN MAMMARY EPITHELIAL CELLS

cell line	transfection	G2/G1 ratio	population doubling time (hr)[a]	saturation density ($x10^5$)[b]
184-B5	none	1.84	32.8 ± 1.6[c]	23.8 ± 2.9[e]
184-B5/HER	HER-2/neu	1.86	21.6 ± 2.1[d]	37.6 ± 7.2[f]

[a] determined from linear portion of the growth curves in log phase cultures.

[b] cell number in ≈ 90% confluent cultures at day 9 post seeding. Initial seeding density: 1.0×10^5 cells per 25 cm^2.

[c-f] mean ± SD n = 6. [c-d, e-f] P = 0.001.

AT-3

DNA ADDUCT FORMATION IN HUMAN MAMMARY EPITHELIAL 184-B5 CELLS

Treatment	Type of DNA adducts (pmole/mg DNA)	
	diolepoxide-purine nucleotide	unidentified
None	ND	0.04
0.1% DMSO	ND	0.08
10 μM DMBA	0.38[a]	ND
10 μM BP	2.06[b]	ND
2 μM EGCG	ND	0.04
10 μM I3C	ND	0.08

ND: not detectable

[a] DMBA diolepoxide-d guanosine + d adenosine

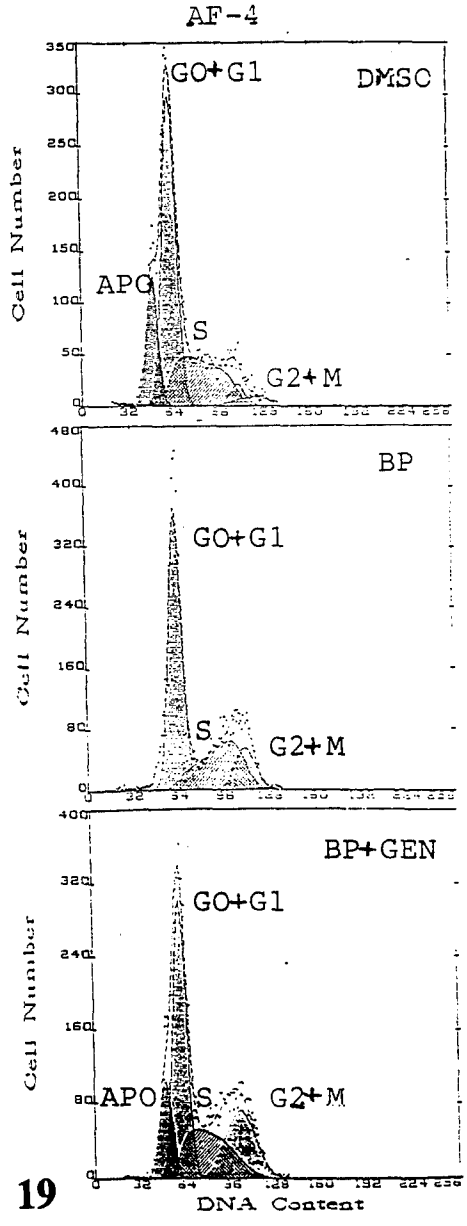
[b] BP diolepoxide - d guanosine + d adenosine

CELL CYCLE ANALYSIS OF HUMAN MAMMARY EPITHELIAL 184-B5 CELLS

Treatment[a]	% Distribution of cells			
	Phase of cell cycle			
	Apo	Go + G1	S	G2 + M
None	17.9	61.6	33.8	4.6
BP	ND	56.2	31.1	12.7
BP + GEN	13.6	46.9	27.7	25.4

[a] 184-B5 cells at ≈ 80% confluence were treated for 24 hr. with BP (39 μM) or with BP + GEN (39 μM + 2.5 μM). Propidium iodide-stained cell suspension was analyzed by FACS.

ND: Not detected.



AT-5

GROWTH INHIBITION IN HUMAN MAMMARY EPITHELIAL CELLS BY PHYTOCHEMICALS

Agent	Concentration	Cell number at day 8 post-seeding [a] ($\times 10^5$)	
		184-B5	184-B5/HER
DMSO (solvent control)	0.1%	21.60 ± 1.90	38.90 ± 0.50
EGCG	2 μM	7.50 ± 1.70 (65.3%)[b]	25.10 ± 2.80 (35.5%)
	22 μM	0.07 ± 0.01 (99.7%)	17.90 ± 1.20 (53.9%)
I3C	10 μM	12.80 ± 0.30 (40.7%)	36.10 ± 0.80 (7.2%)
	100 μM	0.24 ± 0.21 (98.9%)	24.20 ± 1.60 (37.8%)
GEN	2.5 μM	9.70 ± 0.20 (55.1%)	29.80 ± 1.50 (23.4%)
	7.5 μM	0.92 ± 0.08 (95.7%)	18.20 ± 0.70 (53.2%)

[a] mean ± SD, n = 6 per treatment group

[b] % inhibition: $\frac{\text{treated-control}}{\text{control}} \times 100$

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Biomarkers of mammary preneoplastic transformation: end points for cancer chemoprevention

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SUMMARY

In the multistage development of breast cancer initiation and promotion of preneoplasia is an important, early-occurring event representing a marker for risk and for efficacious preventive intervention. In vitro models from non-involved murine and human mammary tissue were utilized to measure i.) upregulation of molecular and endocrine markers in response to treatment with prototype chemical carcinogens and ii.) efficacy of selected naturally-occurring tumor inhibitors in suppressing the perturbed markers. Carcinogen-initiated mammary explant cultures exhibited increased Ras-mediated signal transduction, unscheduled DNA synthesis (UDS) and estradiol 16α-hydroxylation. Treatment of initiated cultures with tumor promoting omega-6 fatty acid increased, while that with tumor inhibiting omega-3-datty acid decreased the perturbed biomarkers. Thus, induction and modulation of biomarkers prior to tumorigenesis validate their clinical relevance as end points for cancer risk and for efficacious preventive intervention.

IN VITRO MODULATION OF HUMAN MAMMARY CARCINOGENESIS: A MODEL FOR CHEMOPREVENTION. Nitin T. TELANG, Division of Carcinogenesis & Prevention, Strang-Cornell Cancer Research Laboratory, Cornell University Medical College, New York, NY/USA

The natural estrogen 17β-estradiol (E_2) influences the mammary cell proliferation, morphogenesis, and neoplastic transformation in vivo. The role of epithelial-stromal interaction in the cellular effects of E_2 is equivocal. Experiments were designed using an in vitro model developed from mammary explants and cell culture to examine whether i.) stromal component affects cellular metabolism of E_2 and ii.) naturally-occurring agents modulate E_2 metabolism. Metabolic conversion of E_2 via C2- and C16α-hydroxylation pathways was monitored by a radiometric assay, and cellular proliferation was measured by ^{3}H-thymidine uptake or by anchorage-independent growth. In the explants of terminal duct lobular units (TDLU) containing inter-lobular and inter-ductal stroma, the chemical carcinogen Benzo(α)pyrene (BP) induced a 164.8% **increase** in E_2 C16α-hydroxylation ($P=0.006$) and a 51.9% **increase** in ^{3}H-thymidine uptake ($P=0.002$). BP treatment also exhibited a 77.6% **decrease** ($P<0.0001$) in E_2 C2/C16α-hydroxylation ratio, which was abrogated ($P<0.0001$) in the presence of eicosapentaenoic acid (EPA) or indole-3-carbinol (I3C). In the cultures of human mammary carcinoma MCF-7 cells EPA and I3C enhanced E_2 C2/C16α-hydroxylation ratio ($P<0.0001$) and inhibited anchorage-independent growth ($P=0.001$). In TDLU and MCF-7 cultures the extent of E_2 C2-hydroxylation was down-regulated ($P=0.005$) by human adipocyte-conditioned medium (h-ACM) obtained from obese subjects. Thus, BP- and h-ACM-induced alteration of E_2 metabolism and its modulation by EPA and I3C in TDLU suggests a paracrine role of stroma in human mammary carcinogenesis. This in vitro model may provide a system to assess the effects of naturally-occurring chemopreventive agents on human mammary cell transformation [Support: Dept. of the Army Grant # DAMD17-94-J-4208, NIH PO1 CA 29502 and the Wanda Jablonski Fund].